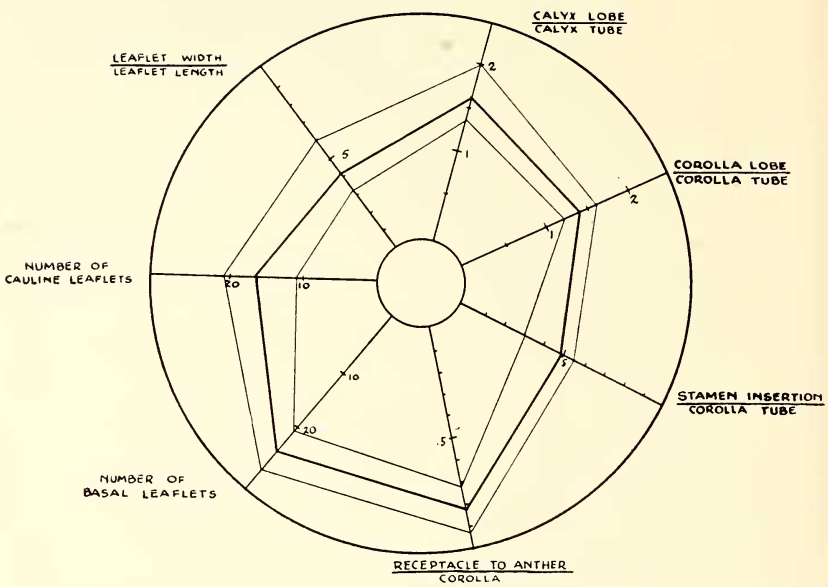

FIG. 6. A synopsis of another *Polemonium* population from the Sierra Nevada of California. The variation from *P. pulcherrimum* is evident in a number of respects in the mean (heavy line), but the calyx proportion appears to be most diagnostic.

———————. 1940. Polygonal graphing of ecological data. Ecology 31: 475–487.

Department of Botany,
University of California, Berkeley.

MATURATION OF THE GAMETES AND FERTILIZATION IN NICOTIANA[1]

T. H. GOODSPEED

Examination of megasporo- and megagametogenesis in a number of species of *Nicotiana* was undertaken with special reference to the extent to which details in the development of the female gametophyte might contribute evidence concerning species origins and relationships. The investigation was later extended to determination of the development, structure and behavior of the sperms. On this latter point no detailed reports have been published in the case of *Nicotiana* and relatively few references to megasporo- or megagametogenesis appear in the literature dealing with the genus.

[1] Contribution no. 118 from the University of California Botanical Garden. Investigations aided by grants of the Committee on Research, University of California.

For *N. tabacum* the complete sequence in megasporogenesis and embryo sac development was investigated with comparative studies, particularly of megagametogenesis, in a number of other species. In *N. alata* early stages were emphasized, in *N. sylvestris, N. glutinosa* and *N. rustica* 2- and 4-nucleate embryo sacs were studied while the character of the 8-nucleate condition in the same three species and in *N. rotundifolia* was compared. In addition, megasporogenesis in certain F_1 hybrids between more and less distantly related species was examined in comparison with that of the species above mentioned.

In *N. tabacum* the archesporial cell differentiating in the subepidermal layer of the nucellus at the apex of the ovule is initially distinguished by its large nucleus, deeply staining cytoplasm and, later, by its increase in volume as contrasted with the surrounding tissue. The archesporial cell becomes the megaspore mother cell directly, without division into parietal and sporogenous cells. Although in *N. tabacum* not more than one megaspore mother cell in an ovule has been seen, in *N. alata* twin megaspore mother cells (pl. 18, fig. 6), and later twin embryo sacs, each covered by its own nucellus, sometimes occur (cf. however, Satina, Blakeslee and Avery, 1934; Rees-Leonard, 1935; Cooper, 1943). In F_1 interspecific hybrids more than one megaspore mother cell has often been observed and the same is true of chromosomal variants, particularly of *N. tabacum,* derived from treatment of sporogenous or vegetative cells with high frequency radiation which, in addition, produced abnormalities in organization of nucellar and other tissues of the ovule.

At the time of differentiation of the megaspore mother cell in *N. tabacum* the ovule is erect but becomes completely anatropous at early meiotic stages. During this inversion epidermal cells at a level just below the lower end of the megaspore mother cell begin by periclinal divisions to form the single integument (pl. 18, fig. 1). It develops rapidly, two or three layers in thickness, and reaches the level of the apex of the nucellus before the end of pachytene, and almost completely covers it by diakinesis-MI (pl. 18, fig. 2).

During meiotic prophases the megaspore mother cell shows rapid increase in size, particularly in length (pl. 18, figs. 1, 2), while the cells of the nucellar covering become exceedingly narrow and elongated (pl. 18, figs. 2–4). At the same time the innermost layer of the integument begins differentiation to form the integumentary tapetum. The two meiotic divisions produce a quartet of megaspores—MII frequently occurring earlier in the chalazal than in the micropylar member of the dyad (pl. 18, fig. 4). More frequently the MII spindles are at right angles to each other producing a T-shaped quartet (pl. 18, fig. 3) although a linear quartet (pl. 18, fig. 4) is not uncommon. The chalazal megaspore becomes the 1-nucleate embryo sac and the other three megaspores soon degenerate (pl. 18, fig. 5).

In *N. tabacum,* therefore, the embryo sac is monosporic (cf. Maheshwari, 1941), as was also reported by Modilewski (1935). However, he found in *N. glauca* "a disporial eight-nucleate embryo sac according to the type of *Scilla,*" and in *Nicotiana ditagla* (amphidiploid *N. tabacum* × *N. glauca*) that "the monosporial process of formation of the embryo sac by means of forming a triad, occupies an intermediate position between the bisporial type which is proper to *N. glauca* and the monosporial type in form of a tetrad distinctive of [*N. tabacum*] Dubeck." In *N. rustica,* Persidski and Modilewski (1934) report that the development of the embryo sac "proceeds according to the disporial or *Scilla* type." Although complete analysis of the early sequence in *Nicotiana glauca* and *N. rustica* has not been made here, it is doubtful whether two distinct types of embryo sac development occur in a single genus. Furthermore, the illustrations and additional comments of the above named authors suggest that the two species in question are not typically bisporic.

Characteristic of the *Tubiflorae,* nucellar degeneration begins during the period of the extremely rapid growth in volume of the increasingly vacuolate 1-nucleate embryo sac while the remnants of the three non-functional megaspores are still present (pl. 18, fig. 5). This degeneration proceeds rapidly so that little indication of nucellar tissue is seen at the 2- and 4-nucleate stages, and almost none at the 8-nucleate stage (pl. 18, fig. 7).

Following the establishment of the 8-nucleate condition, one each of the four nuclei at the antipodal and at the micropylar end moves toward the center of the embryo sac. During this period the egg apparatus and the antipodal cells are matured. The former consists of triangular or pear-shaped synergids provided with basal vacuoles and a somewhat more spherical egg which continues to enlarge as a result of increase in size of an upper vacuole.

While in *N. tabacum* and *N. glutinosa* the mature embryo sac is pointed at the micropylar end and rounded at the chalazal end

---

Explanation of the Figures. Plate 18

Plate 18. Some Stages in Megasporogenesis and Embryo Sac Formation in Nicotiana. Figs. 1–4. Megasporogenesis in *Nicotiana tabacum.* Fig. 1. Megaspore mother cell, early prophase; origin of integument. Fig. 2. Same, diakinesis. Fig. 3. T-shaped quartet of megaspores. Fig. 4. Linear quartet; chalazal megaspores already formed, MII in upper dyad cell. Fig. 5. *N. tabacum.* 1-nucleate embryo sac; degeneration of other three megaspores advanced, of nucellus beginning. ×375. Fig. 6. *N. alata.* Twin nucelli, each with a megaspore mother cell. Fig. 7. *N. tabacum.* Mature embryo sac, normal organization of 8-nucleate condition, remnants of nucellus, chalazal end rounded. ×375. Fig. 8. *N. rotundifolia.* Same, chalazal end pocketed. ×375. Fig. 9. F_1 *N. tabacum* × *N. glauca.* Dyad degenerating; enlargement of cells of nucellar epidermis within integumentary tapetum (cf. fig. 4). All figures drawn with the camera lucida by a special carbon pencil technique from paraffin sections of ovules—longitudinal; reproduced ×290 unless otherwise indicated.

PLATE 18. SOME STAGES IN MEGASPOROGENESIS AND EMBRYO SAC FORMATION IN NICOTIANA.

(pl. 18, fig. 7), in *N. rustica* both ends are pointed; in *N. rotundifolia* (pl. 18, fig. 8)—to a lesser degree also in *N. sylvestris*—the chalazal end is distinctly pocketed. Although in the other species examined distinctions in the structure of the included cells serve to distinguish the two ends of the embryo sac, in *N. rotundifolia* two antipodals are similar to the synergids and the third antipodal suggests the egg. Attention is called to the fact that the species just referred to as differing in shape of embryo sac are members of three distinct taxonomic subdivisions of the genus. The extent to which such variations possess phylogenetic significance must, however, await further investigation.

Examination of megasporogenesis was made in a number of F_1 interspecific hybrids. In most cases the megaspore mother cell degenerates during meiosis, in some after the first and in others during the second division; the 4-megaspore stage was observed only in F_1 *N. bigelovii* × *N. suaveolens*. As degeneration proceeds the surrounding nucellar cells increase in volume (pl. 18, fig. 9) for a considerable period to produce a condition in striking contrast to that in species where, as already noted, the nucellar covering shows a progressive decrease in cell volume (cf. Greenleaf, 1941).

The sequence leading to the maturation of the female gametophyte, as summarized above, was determined from paraffin sections of *N. tabacum*[2] prepared by conventional techniques. Sections of entire ovaries from which the wall had been removed were cut at 15–20 μ and stained in iron haematoxylin. The following discussion of the origin, morphology and behavior in fertilization of the male gametes is based upon analysis of squash and smear preparations of *N. tabacum*[3] and of *N. longiflora* produced by a technique described elsewhere by Dr. Muriel V. Bradley (1948). By the use of this remarkably effective technique which permits examination of entire embryo sacs preceding and following fertilization many of the foregoing observations have been confirmed (cf., also, photomicrographs, Bradley, 1948).

In *Nicotiana* cytokinesis by furrowing originates the quartet of immature microspores which become the elliptical pollen grains. The mitosis producing the vegetative and generative nuclei is approximately central rather than near the wall of the microspore (Brumfield, 1941), apparently because of the absence of a central vacuole (Sax, 1935).

Normally in *Nicotiana* the division of the generative nucleus occurs in the pollen tube although under conditions of artificially induced germination this mitosis is sometimes observed in the pollen grain itself. Pollen tube mitoses have been studied in *N.*

[2] A variety collected, as an escape from cultivation, near Sartimbamba, northern Peru. The taxonomic status of other species referred to here is commented upon elsewhere (Goodspeed, 1945a).

[3] A variety collected, as an escape from cultivation, near Huaras, Peru.

tabacum, N. longiflora and *N. otophora.* Distinctions in chromosome morphology within the genoms of these species are clearly seen in these mitoses. Thus, for example, in *N. otophora* the five long *st* and the seven short *m* chromosomes (Goodspeed, 1945b) can be distinguished (pl. 19, fig. 5). Examination of pollen tubes of *N. tabacum* at increasing distances from the stigma indicates that the division of the generative nucleus characteristically occurs in the upper third of the style.

From dissections and smears of the stigma and style of *N. tabacum* it is clear that before pollen tube development the vegetative nucleus may exhibit marked alteration in form and substance. At first, its outline becomes irregular and its contents somewhat diffuse and weakly staining (pl. 19, fig. 1). Later, as it advances in the tube its substance is greatly extended. As shown in plate 19, figure 3, its material becomes thrown into folds or loops and even, at times, a long, twisted ribbon commonly terminating, at one or both ends, in a more condensed region. Its extension may be rather extraordinary (pl. 19, fig. 2). Following

---

Explanation of the Figures. Plate 19

Plate 19. Maturation of the Male Gametes and Fertilization in Nicotiana. Figs. 1–4, 6–16. *N. tabacum,* n = 24; Fig. 5. *N. otophora,* n = 12; Figs. 17–22. *N. longiflora,* n = 10. Fig. 1. Pollen grain from stigma, early alteration in form of vegetative nucleus. ×180. Figs. 2–4. Portions of pollen tubes from stylar canal. ×290. Fig. 2. Extreme thread-like form of vegetative nucleus. Fig. 3. More usual appearance of thread-like vegetative nucleus; late prophase of generative nucleus. Fig. 4. Later condensation of vegetative nucleus; anaphase of generative nucleus. Fig. 5. Division of generative nucleus in pollen tube, 5 large subterminal and 7 smaller median chromosomes. ×850. Fig. 6. Portion of pollen tube in micropyle; further condensation of vegetative nucleus (cf. figs. 2–4); sperms. Figs. 7–22. Studies of embryo sacs during and following fertilization (for details, cf. text). Figs. 7–9. Sperms, discharged from pollen tube, before contact with egg and larger fusion nucleus, sperms earlier elongated (figs. 7, 8), later spherical (fig. 9); remnant of vegetative nucleus, tapering, pycnotic; degenerating nucleus of disrupted synergid ring-like; other synergid below pollen tube cytoplasm; sperm cytoplasm apparent (fig. 9). Figs. 10–12, 18, 19, 21. Early, mid- and late fertilization stages, sperms undergoing alteration in form and structure; incorporation of sperm in fusion nucleus more rapid; tapering vegetative nucleus and one synergid pycnotic, other synergid intact; cytoplasm of early zygote differentiating (figs. 12, 21); cytoplasm of sperms apparent (fig. 11). Figs. 13, 14. Early and late fertilization, before fusion of polar nuclei; differentiation of zygote cytoplasm conspicuous. Fig. 15. Entire embryo sac, late fertilization stage with incorporation of sperms almost complete; cellular character of antipodals. Fig. 16. Metaphase of first division of primary endosperm nucleus, *N. tabacum,* 72 chromosomes. Fig. 17. Contents of two pollen tubes in embryo sac, post fertilization; sperms from second pollen tube near zygote and primary endosperm nucleus; pycnotic degeneration products of two vegetative nuclei (one above zygote) and two synergids. Figs. 20, 22. Post fertilization, persistence of vestiges of vegetative nucleus and one synergid nucleus, other synergid intact; differentiation of zygote cytoplasm; metaphase and telophase of first division of primary endosperm nucleus, *N. longiflora,* 30 chromosomes (fig. 20). All figures drawn with the camera lucida by a special carbon pencil technique from squash or smear preparations; reproduced ×375 unless otherwise indicated.

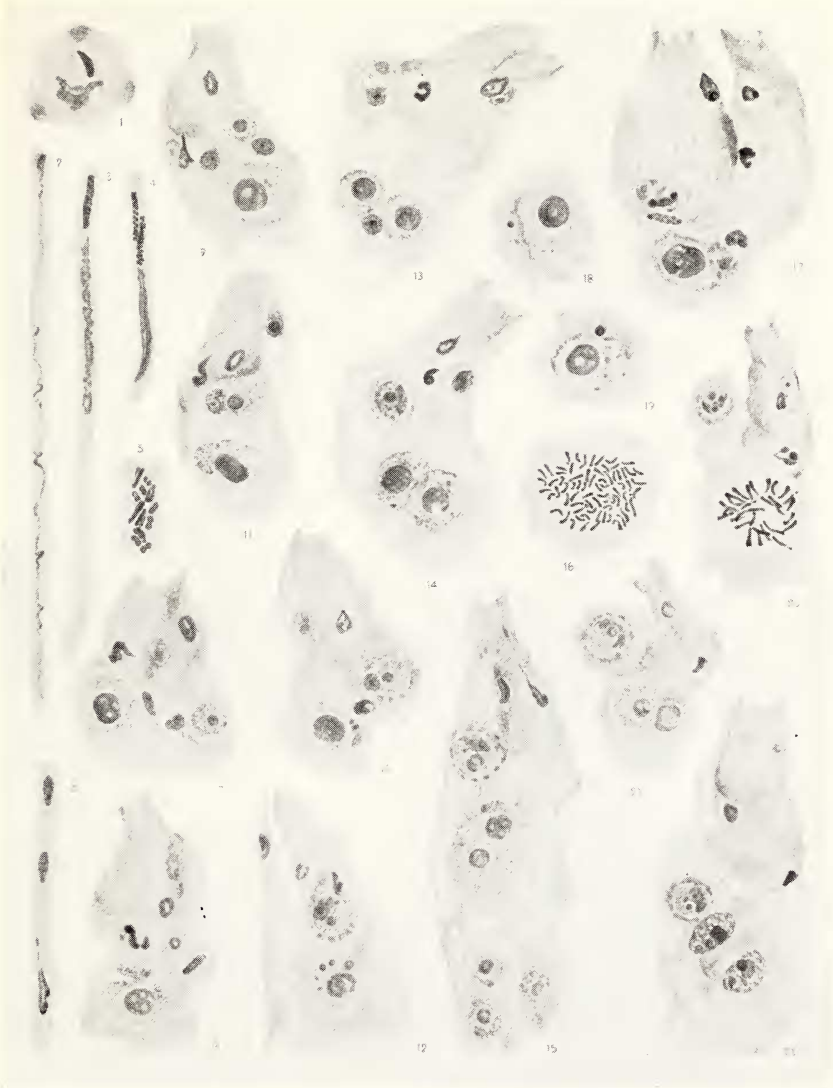

PLATE 19. MATURATION OF THE MALE GAMETES AND FERTILIZATION IN NICOTIANA.

the division of the generative a certain condensation of the material of the vegetative nucleus occurs (pl. 19, fig. 4), which continues with increasing evidence of pycnosis as the tube enters the micropyle (pl. 19, fig. 6). A somewhat tailed, deeply staining structure represents an advanced stage in the degeneration of the vegetative nucleus. Variations in its appearance at fertilization and somewhat later are shown in plate 19, figures 7 to 15, and the fact that it persists at least until the primary endosperm nucleus is in division appears in plate 19, figures 20 and 22.

Numerous investigators (cf. Schnarf, 1941) have noted in other genera, and variously interpreted, similar changes in the form of the vegetative nucleus from the large, irregular, amoeboid, weakly staining condition characteristic of the pollen grain to the much extended, often almost thread-like form assumed in the tube. The change from an amoeboid to an extended form may be a response to protoplasmic streaming and the autonomous movement of the vegetative nucleus in the narrow confines of the tube (Tischler, 1925; O'Mara, 1933). Earlier literature attributes to the vegetative nucleus initiation of tube development followed by degeneration. More recent interpretations agree in questioning early degeneration and tentatively assign to the vegetative nucleus a function related to the continued growth of the tube (Schnarf, 1941; cf., however, Poddubnaja-Arnoldi, 1936). In *Nicotiana* degeneration as indicated by pycnosis is conspicuous only after growth of the pollen tube is at an end and, certainly, as suggested by Wulff (1933), if the vegetative nucleus is the bearer of growth-promoting substances any increase in its surface would have causal significance.

Studies of smears and squashes of styles and ovules of an horticultural race of diploid *Petunia* show, in this genus closely related to *Nicotiana,* a sequence of events in the development and degeneration of the vegetative nucleus corresponding to that in *Nicotiana.* However, in *Petunia* this nucleus is commonly more weakly staining and its irregularity and elongation are less conspicuous than in *Nicotiana.* Correspondingly, evidence of its degeneration is not conspicuous until after fertilization, by contrast with a strikingly pycnotic degeneration product of this nucleus which in *Nicotiana* is found along with the two sperms, in the tube cytoplasm previous to its contact with the female nuclei.

The proper squash technique applied to ovules provides an abundance of material for study of the sequence beginning with the penetration of the micropyle by the pollen tube and continuing through the divisions of the endosperm and zygote nuclei. Although the evidence thus obtained corresponds in general to that reported by Guignard (1902) for *N. tabacum,* a considerable amount of additional information is now available. Also, various stages in fertilization have for the first time been seen in species of *Nicotiana* other than *N. tabacum.*

Normally and as noted above, the 8-nucleate embryo sac of the species of *Nicotiana* investigated becomes the seven celled megagametophyte; the differentiation of synergids, egg and antipodals is accompanied by wall formation (cf. Bradley, 1948). Vacuolation produces a broad band of cytoplasm connecting egg, fusion nucleus and antipodals, with strands extending to the walls of the embryo sac. In at least one race of *N. tabacum* numerous deviations from such normal development occur (cf. Bradley, 1948; Persidski and Modilewski, 1934, in *N. rustica*). Thus, 9- to 16-nucleate embryo sacs have been seen, obviously the result of division of from one to all of the normal eight nuclei (cf., also, Korotkevich, 1940, in *N. rustica*). Frequently, alterations in normal position of nuclei, and particularly antipodal ones, occur. From one to three antipodals may wander to a position near the egg so that in some instances all nuclei are found in the micropylar end of the embryo sac (cf. Guignard, 1902).

Although polar fusion is usually complete before fertilization, it is in progress thereafter too frequently to be classed as an abnormality. Indeed, Guignard (1902) considered post fertilization fusion of the two polar nuclei normal behavior in *N. tabacum*. Fusion of three nuclei in 8-nucleate embryo sacs and of from three to five in the multinucleate embryo sacs above described has been observed. Twin embryo sacs have been found in squash preparations, with the corresponding nuclei similarly disposed and the two embryo sacs in identical stage of development.

In *N. longiflora,* and presumably in other species also, two pollen tubes may penetrate the micropyle. The contained cytoplasm of two tubes each with two sperms, a pycnotic vegetative nucleus and a degenerating synergid nucleus may be seen in contact with the female nuclei. On the other hand, penetration may not be simultaneous for, as shown in plate 19, figure 17, fertilization of both the egg (seen under the mass of tube cytoplasm) and fusion nucleus has apparently been effected by the sperms of the tube first penetrating the embryo sac while those of the second tube appear in the combined cytoplasm and presumably will later degenerate there.

In those species of *Nicotiana* studied the male gametes undergo little structural alteration from the time of their origin in the pollen tube until their contact with the female nuclei, and apparently the same is true of *Petunia.* Throughout they are somewhat elongated, ovoid bodies (pl. 19, figs. 6, 7; cf. Bradley, 1948, fig. 8). However, it is not possible to confirm the conclusions of Poddubnaja-Arnoldi (1936) and Sarana (1934) for *Nicotiana* nor the suggestion of Cooper (1946) for *Petunia* that the sperms in the pollen tube are cells. On the other hand, in both genera, and particularly in the latter, in squash preparations one sperm apparently within the egg cell and the other near or in contact with the fusion nucleus have been observed, each surrounded by a cyto-